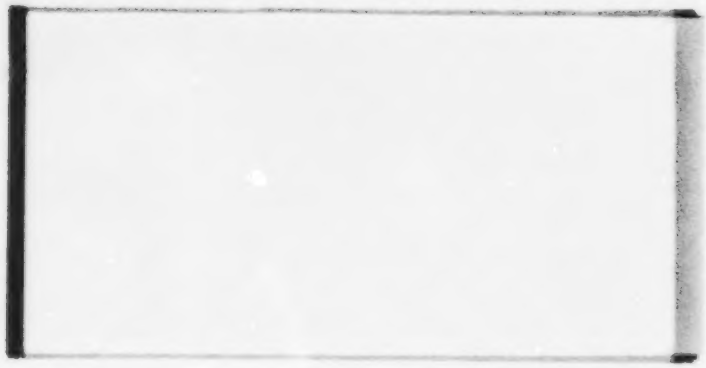
Ontario Hydro



RESEARCH DIVISION

91602
new 82-10

ontario hydro
research division

COMPLIMENTARY COPY
Please Credit Ontario Hydro Research if
Reproduced in Whole or in Part.

THE INFLUENCE OF VARIED TEMPERATURE REGIMES ON THE DEVELOPMENT OF RAINBOW TROUT EMBRYOS

Report No 85-337-K

R.S. McKinley
Biologist
Biological Research Section
Chemical Research Department

ABSTRACT

The concept of retardation and control of hatching in rainbow trout embryos by varying incubation temperatures was investigated. Systematically varying temperature (increasing or decreasing) during pre-determined embryonic periods resulted in the doubling of time to hatch. Results also indicated that maintaining incubation temperature at less than four degrees C before eyeing caused 95% mortality of the embryos. Maintaining incubation temperature above four degrees C until eyeing and then reducing the incubation temperature to less than four degrees C dramatically improved survival (60%), yet allowed development times to be approximately doubled.

job	file	date	report no.
740634-510-011	836.4	November 26, 1985	85-337-K

91602
new 82-10

ontario hydro
research division

TO: Mr. F.J. Kee
Director of Research

THE INFLUENCE OF VARIED TEMPERATURE REGIMES ON THE DEVELOPMENT OF RAINBOW TROUT EMBRYOS

INTRODUCTION

This study is part of an investigation on thermal aquaculture. Information is presented on the thermal control of rainbow trout (Salmo gairdneri) embryos as a method for overcoming the problem of seasonality which imposes some limits on the availability of fish on a year-round basis. A means of ensuring year-round production of stocking-size fish at low cost is being investigated. Typically, adult fish have to be maintained on a year-round basis and through the manipulation of photoperiod reproduction is encouraged at different times of the year. This process is costly and not overly effective. The alternative that was investigated is the retardation and control of hatching in rainbow trout embryos by varying the incubation temperature during embryonic development.

The effect of temperature on the duration of salmonid incubation and tolerance ranges has been intensively studied (Swift 1965, Hokanson et al. 1973, Crisp 1981). Surprisingly few studies have been performed that describe the effects of temperature variation from fertilization through embryonic development on hatch success or mathematically describe the functional relationship between varying temperatures and the duration of egg development (Peterson et al. 1977, Jungwirth and Winkler 1984).

The objective of this study was to determine the effects of systematic temperature variation on the development of rainbow trout embryos. Specific aspects considered were the effects of temperature variation on embryo mortality, time from fertilization to the occurrence of eye pigment, time from eye pigment to pre-hatch and pre-hatch to hatch.

METHOD AND MATERIALS

Eggs used were obtained from the Ganaraska Fishway on April 10, 1984. The eggs from two ripe females were fertilized with the milt from two ripe males, and immediately transported to incubation apparatus at the Dobson Research Laboratory.

job	file	date	report no.
740634-510-011	836.4	November 26, 1985	85-337-K

The egg incubation apparatus consisted of 48 vertical cylinders of clear acrylic and PVC (Ontario Hydro Research Division Report No 78-209-K). One hundred and fifty embryos were incubated in PVC chambers with 363 u Nitex screen at the top and bottom. A chamber was placed near the bottom of each cylinder. Dechlorinated Toronto tap water was supplied from constant temperature head tanks. Water entered from the bottom and exited at the top of each cylinder, providing good temperature control and, as well, maintaining dissolved oxygen levels at or near saturation. Temperatures for each treatment were obtained by adjusting the flow from head tanks. Light conditions simulated the natural photoperiod.

Experimental Design

Two basic concepts were tested. Experiment 1 involved increasing temperatures at pre-selected embryonic stages during development whereas the opposite was true for Experiment 2.

Experiment 1

A factorial design was used with four base incubation temperatures, and increases at two stages of development. Base incubation temperatures were 1, 3, 5, and 7°C. When approximately 100% of the embryos had developed eye pigment at a particular temperature, a subset of three incubation chambers was placed at one of the higher base temperatures. Similarly, when 50% of these eyed embryos had reached prehatch a subset of three incubation chambers was placed at one of the higher base temperatures. A set of three incubation chambers remained at each of the four base temperatures throughout the experiment (Figure 1). The effects of systematically varying temperature on the rate of development and mortality among the periods of fertilization to eyeing (T1), eyeing to pre-hatch (T2) and pre-hatch to 50% hatch were noted (T3).

Experiment 2

A factorial design as above was used, except that the embryos were placed at only two base temperatures: 7 and 5 degrees C. Preliminary results indicated that temperature sensitivity was highest during the first stages of development (T1). Incubations through to formation of eye pigment (T1) were maintained at 7°C and 5°C. At completion of eyeing, a subset of these embryos was placed at a lower temperature until completion of 50% hatch (T2+T3) (Figure 1). Again, the rate of development and mortality for each period were noted. Temperatures were recorded continuously for each incubation chamber. Embryo development was monitored and recorded weekly, using the developmental stages outlined by Garside (1959). Egg mortality in each incubation chamber was noted and dead eggs

were removed to prevent fungal growth. Newly hatched fry were examined for abnormalities. Both experiments were continued until all viable eggs had hatched.

Attempts were made to maintain temperatures within $\pm 1^{\circ}C$; however, due to equipment malfunction during the eye to pre-hatch period of the first experiment, the one and three-degree base temperatures were not achieved. These averaged 1 to $3^{\circ}C$ higher than the target temperature. Development time and mortality were noted for the actual temperature regime.

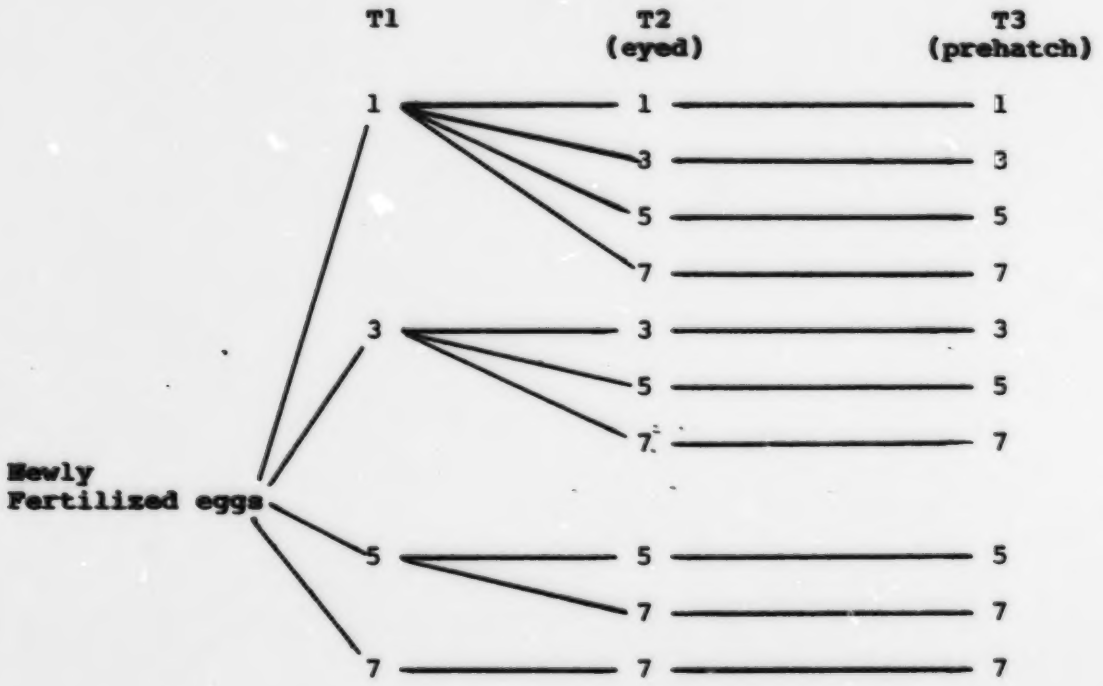


Figure 1. Schematic representation of the design for experiment 1. T1 represents the incubation temperature during the embryonic period of fertilization to eyeing. T2 and T3 represents the respective second and third increase in incubation temperature at pre-selected embryonic stages (eyed and pre-hatch respectively).

RESULTS

In the first experiment, total mortality occurred at the coldest base temperature (1.6 - 2.0 degrees C) and hence data were omitted from further analysis.

Mortalities at the three-degree base temperature were extremely severe, in excess of 90% in all sets (Table 1), during the first embryonic period (fertilization to eyeing). At the five, and to a much lesser extent at the seven-degree base temperatures, mortalities during the first period were greatly reduced but highly variable.

In the second experiment, the initial embryonic period (T1) was maintained under higher temperatures then subsequently reduced for the eyeing to hatch period. Following this procedure mortalities were greatly reduced during the initial period (<20%) (Table 1). Only when incubation temperatures were reduced to three and one degree C during T2+T3 (eyeing to hatch) did mortality dramatically increase and in most cases more than doubled. Overall, however, maintaining the T1 at five or seven degrees C until embryos eyed resulted in an approximately 50 to 60% improvement in survival at hatch.

All individuals maintained at a three-degree base temperature for the first embryonic period were malformed at hatch and died within forty-eight hours following hatch. Malformations included individuals with two heads, multiple eyes and sharing a common tail. Individuals maintained at temperatures >3 degrees C during the first embryonic period survived for at least 96 h following hatch.

As expected, embryo development times were indirectly related to incubation temperature (Table 2). Maximum and minimum development times from fertilization through to hatch averaged 103 days for embryos incubated under the coldest viable temperature regime (3-5-3) to 56 days for eggs incubated at the warmest temperature regime (7-7-7). Development time for the initial embryonic period ranged from 29 at seven degrees C to 69 days at three degrees C. Time for the second period ranged from 17 to 27 days for 5/6 degrees C with those from a three base temperature having a shorter developmental period than those from the five-degree base. At seven degrees, time ranged from 7 to 21 days and again with the ones from the lowest base temperature having the shorter development time. Time of the last period ranged from 7 to 11 days for both six and seven-degree C incubation temperatures. Overall, the main impact of the base temperature on embryonic development was evident during the first step where development at three degreesC was more than twice that at seven degreesC, in subsequent stages (T2+T3), the impact was substantially less and often reversed.

TABLE 1

PERCENT MORTALITY FOR EACH DEVELOPMENT PERIOD UNDER VARYING TEMPERATURE REGIMES. STANDARD DEVIATIONS ARE IN PARENTHESES. RECORDED TEMPERATURES ARE BRACKETED.

EXPERIMENT 1

	3-3-3 [3-5-3]	3-3-5 [3-6-5]	3-3-7 [3-6-7]	3-5-5 [3-6-7]	3-7-7 [3-7-7]
*STAGE					
T1	95.22 (2.7)	93.63 (2.2)	95.70 (3.6)	94.88 (2.7)	91.90 (3.8)
T2	0.00	2.56 (3.1)	3.34 (2.7)	2.38 (2.9)	0.00
T3	0.00	11.11 (9.0)	0.00	2.56 (3.1)	0.00
TOT	95.22 (2.2)	97.30 (2.2)	90.60 (3.4)	98.14 (2.2)	91.90 (3.8)

	5-5-5 [5-5-6]	5-5-7 [5-5-7]	5-7-7 [5-7-7]
T1	7.83 (9.2)	0.24 (0.3)	1.37 (0.9)
T2	0.81 (0.6)	0.45 (0.3)	0.23 (0.3)
T3	1.44 (0.4)	1.88 (0.8)	0.24 (0.3)
TOT	9.87 (2.1)	3.47 (0.8)	2.06 (1.5)

	7-7-7 [7-7-7]
T1	0.00
T2	0.00
T3	0.45 (0.5)
TOT	0.45 (0.5)

* T1-T2-T3°C correspond to the developmental periods; fertilization to eyeing, eyeing to pre-hatch, and pre-hatch to hatch.

EXPERIMENT 2

*STAGE	7-7 [7-7]	7-5 [7-5]	7-3 [7-3]	7-1 [7-1]
T1	8.5 (5.3)	0.6 (0.4)	2.0 (0.7)	2.8 (1.9)
T2+T3	3.6 (0.4)	5.6 (0.8)	11.3 (2.0)	34.3 (2.8)
TOT	13.1 (1.4)	6.3 (1.7)	14.0 (0.4)	36.0 (11.4)

	5-5 [5-5]	5-3 [5-3]	5-1 [5-1]
T1	18.0 (2.8)	18.0 (3.8)	20.6 (5.7)
T2+T3	1.8 (1.4)	37.3 (5.6)	23.4 (4.7)
TOT	22.0 (3.7)	59.1 (3.1)	49.9 (3.6)

* T1-T2-T3°C correspond to the developmental periods; fertilization to eyeing, eyeing to pre-hatch, and pre-hatch to hatch.

TABLE 2

MEAN TIME (DAYS) FOR EACH DEVELOPMENT PERIOD UNDER VARYING TEMPERATURE REGIMES. STANDARD DEVIATION IS IN PARENTHESES. RECORDED TEMPERATURES ARE BRACKETED.

EXPERIMENT 1

	3-3-3 [3-5-3]	3-3-5 [3-6-5]	3-3-7 [3-6-7]	3-5-7 [3-6-7]	3-7-7 [3-7-7]
*STAGE					
T1)	69 (0)	69 (0)	69 (0)	69 (0)	69 (0)
T2)	17 (0)	17 (0)	17 (0)	9 (0)	7 (0)
T3)	17 (0)	11 (0)	7 (.7)	15 (.8)	13 (1.7)
TOT	103 (0)	97 (0)	93 (.8)	93 (.8)	89 (1.7)

	5-5-5 [5-5-6]	5-5-7 [5-5-7]	5-7-7 [5-7-7]
T1)	37 (0)	37 (0)	37 (0)
T2)	27 (0)	27 (0)	21 (0)
T3)	11 (1.2)	7 (0)	10 (1.6)
TOT	75 (1.2)	71 (0)	68 (1.6)

	7-7-7 [7-7-7]
T1)	29 (0)
T2)	16 (0)
T3)	11 (.8)
TOT	56 (.8)

* T1,T2 and T3 respectively correspond to the incubation regime for each of the three embryonic periods - fertilization to eyeing, eyeing to pre-hatch, and pre-hatch to hatch.

EXPERIMENT 2

	7-7 [7-7]	7-5 [7-5]	7-3 [7-3]	7-1 [7-1]
*STAGE				
T1)	35 (0)	35 (0)	35 (0)	35 (1.7)
T2+T3)	21 (0)	25 (0)	36 (.7)	62 (3.3)
TOT	56 (0)	60 (0)	71 (.8)	97 (2.1)

	5-5 [5-5]	5-3 [5-3]	5-1 [5-1]
T1)	45 (0)	45 (0)	45 (2.1)
T2+T3)	30 (0)	43 (0)	63 (3.5)
TOT	75 (0)	88 (0)	102 (5.3)

* T1,T2 and T3 respectively correspond to the incubation regime for each of the three embryonic periods - fertilization to eyeing, eyeing to pre-hatch, and pre-hatch to hatch.

Temperature "optimum"

The optimum temperature combination among the three embryonic periods is defined here as one where total development time is maximized with minimum mortality. The relationship between total development time and mortality under various incubation temperature combinations is shown in Figure 2.

An approximate doubling of total development time between the coldest and warmest incubation temperatures used during this study was achieved by maintaining the initial embryonic period at the lowest base temperature and then subsequently increasing the temperature for the remaining two periods. Similar results were obtained if the temperature sequence was reversed. Embryo survival until hatch, however, was noticeably different between the two methods for increasing development time. Total mortality appeared to exponentially increase as the initial incubation temperature decreased. Maintaining the initial embryonic period (fertilization to eyeing) at 5 or 7 degrees C followed by decreasing temperatures in later stages not only resulted in a doubling of total development time but also in an approximately 60% improvement in embryo survival at hatch.

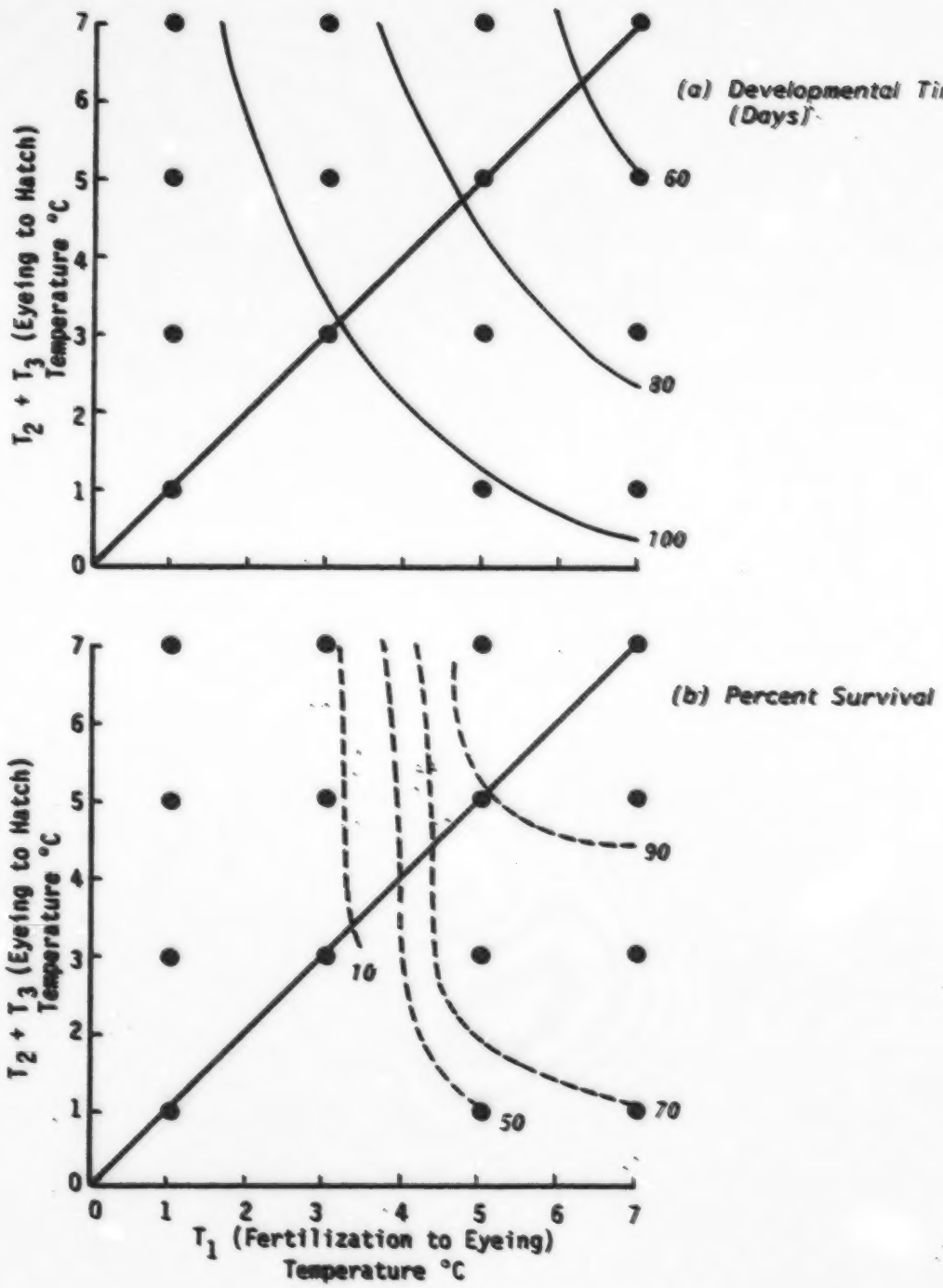


FIGURE 2

CONTOUR LINES OF EQUAL TIME (DAYS) FROM FERTILIZATION TO HATCH (SOLID LINES) AND FOR PER CENT SURVIVAL (DASHED LINES) ARE SHOWN FOR VARIOUS COMBINATIONS OF T_1 AND $T_2 + T_3$

169971-RD

DISCUSSION

The effect of initially incubating recently fertilized rainbow trout eggs at three degrees C compared to seven degrees C resulted in a ≈100% extension in development time. However, accompanying the dramatic increase in development time was an equally dramatic increase in embryo mortality (to 95%).

The majority of the newly fertilized rainbow trout eggs maintained at <4 degrees C did not survive epiboly. This sensitivity of embryos to temperatures of less than four degrees C prior to eye pigmentation has been noted for Atlantic (Salmo salar) (Peterson et al. 1977), Sockeye (Oncorhynchus nerka), Chinook (O. tshawytscha) (Combs 1965) and Pink salmon (O. gorbuscha) (Combs and Burrows 1957). Splake (Salvelinus fontinalis X S. namaycush) for instance, has been shown not only to be sensitive during epiboly but also at pre-hatch stages (Ontario Hydro Research Division Report 78-209-K). The increase in percent mortality with decreasing temperature during T3 (pre-hatch to hatch) suggested that rainbow trout embryos may also share another similar sensitive period.

The data further indicated that systematically increasing or reducing incubation temperature during developmental periods results in a similar extension in total development time. A significant reduction in overall mortality (60%) however, did result when embryos were initially maintained at >4 degrees C until eyed and then reduced to temperatures <4 degrees C for the remaining two developmental periods. None of the individuals that hatched were malformed.

Because the sensitivity to low temperatures occurred at a much earlier stage than eyed suggests that development of newly fertilized eggs may be further extended without dramatically increasing mortality. Combs (1965) for instance, found that for Pacific salmon, embryos were more tolerant of low temperatures beyond the 128-cell stage. Transferring rainbow trout embryos to less than four degrees C at just beyond epiboly would result in an approximately one month extension in developmental time over that achieved here.

In summary, the optimal temperature regime for the purpose of increasing the availability of embryos is one in which the initial embryonic period is maintained at 7 degrees C and the latter two periods at <4 degrees C. The percent survival in this regime more than compensates for the greater developmental time when the first embryonic period is maintained at 5 degrees C then reduced to <4 degrees after eyeing. Although rainbow trout are basically spring spawners (Scott and Crossman 1973) eggs are available from commercial growers in the fall. Subjecting embryos from both

spring and fall spawners to the above temperature regime would ensure the availability of embryos and therefore alevins for stocking on a year-round basis.

CONCLUSIONS

1) Systematically varying the temperature (ie, increasing or decreasing) among the developmental periods resulted in a similar extension in time.

2) Maintaining newly fertilized eggs at less than four degrees C before eyeing resulted in greater than ninety-five percent mortality.

3) Incubation of embryos at greater than four degrees C before eyeing and then reducing the temperature to less than four degrees C resulted in a 60% improvement in survival (compared with low-temperature incubation during the early development stages).

4) The optimal temperature regime for the purpose of producing alevins for stocking on a year-round basis was maintaining the initial developmental period at seven degrees C until eyeing and then reduce the incubation temperature to less than four degrees until hatching.

5) The concept of retarding and control of hatching in rainbow trout embryos by varying incubation temperature was viable with successful hatching times more than doubled.

Approved by:

O.A. Kupcis
Manager
Chemical Research Department

Prepared by:

R.S. McKinley
Biologist
Biological Research Section

Reviewed by:

G.L. Vascotto
Supervising Biologist
Biological Research Section

RSMcK:rv

LITERATURE CITED

1. **Combs, B.D.** 1965. Effect of temperature on the development of salmon eggs. Prog. Fish Cult. 27:134-137.

2. **Combs, B.D. and R.E. Burrows.** 1957. Threshold temperatures for the normal development of chinook salmon eggs. Prog. Fish Cult. 19:3-6.

3. **Crisp, D.T.** 1981. A desk study of the relationship between temperature and hatching time for eggs of five species of salmonid fishes. Freshwater Biol. 11:361-368.

4. **Hokanson, K.E.F., J.H. McCormick, B.R. Jones and J.H. Tucker.** 1973. Thermal requirements for maturation, spawning and embryo survival of the brook trout (Salvelinus fontinalis). J. Fish Res. Bd. Can. 30:975-984.

5. **Jungwirth M. and H. Winkler.** 1984. The temperature dependence of embryonic development of grayling (Thymallus thymallus), Danube salmon (Hucho Hucho), Arctic char (Salvelinus alpinus) and brown trout (Salmo trutta fario). Aquaculture 38:315-327.

6. **Ontario Hydro Research Division Report 78-209-K.** Effects of fluctuating temperatures on splake eggs - potential effects of thermal discharges. J.S. Griffiths.

7. **Peterson, R.H., H.C.E. Spinney and A. Sreedharan.** 1977. Development of Atlantic salmon (Salmo salar) eggs and alevins under varied temperature regimes. J. Fish Res. Bd. Can. 34:31-43.

8. **Scott, W.b. and E.J. Crossman.** 1973. Freshwater Fishes of Canada. Fisheries Res. Bd. Can. Bulletin 184. 966 pp.

9. **Swift, D.R.** 1965. Effect of temperature on mortality and rate of development of the eggs of the Windermere Char (Salvelinus alpinus). J. Fish Res. Bd. Can. 22(4):913-917.